



# Feline immunodeficiency virus subunit vaccines that induce virus neutralising antibodies but no protection against challenge infection

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*Three experimental vaccines against feline immunodeficiency virus (FIV), all based on viral antigens presented via immune stimulating complexes (iscoms), were tested for their capacity to induce protection in cats from FIV infection. The respective vaccines consisted of FIV propagated in Crandell feline kidney (CrFK) cells (FIV-iscoms); FIV-iscoms spiked with recombinant vaccinia virus expressed FIV envelope glycoprotein incorporated into iscoms (FIV-iscoms+vGR657x15-iscoms) and vGR657x15-iscoms spiked with recombinant FIV Gag protein incorporated into iscoms (vGR657x15-iscoms+FIV-Gag-iscoms). Simian immunodeficiency virus envelope glycoprotein incorporated into iscoms, iscoms prepared with uninfected CrFK cells, and PBS served as controls. All cats vaccinated with vGR657x15-iscoms combined with FIV-iscoms or FIV-Gag-iscoms developed Env-specific plasma antibody responses. These antibodies neutralised FIV infection in CrFK cells, but failed to neutralise FIV infection in primary feline thymocytes. FIV-iscoms induced poor Env-specific responses and only one out of six cats developed antibodies that neutralised FIV in the CrFK cell based assay. Four weeks after challenge all cats proved to be infected, showing that none of the vaccine preparations provided protection. In contrast, 2 weeks after infection, virus infected peripheral blood mononuclear cells were only observed in cats vaccinated with FIV-iscoms+vGR657x15-iscoms or CrFK-iscoms and to a lesser extent in cats vaccinated with FIV-iscoms and vGR657x15-iscoms+FIV-Gag-iscoms, but not in cats vaccinated with SIV-iscoms or PBS. The differences found in cell associated virus loads amongst the respective groups are discussed in the light of antibody mediated enhancement of infectivity and protective effects provided by Gag-specific T cell responses. © 1997 Elsevier Science Ltd. All rights reserved*

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Feline immunodeficiency virus (FIV) is a T-lymphotropic lentivirus that causes immunodeficiency syndrome in cats similar to AIDS in humans. Since FIV and human immunodeficiency virus (HIV) share several characteristics and show a similar pathogenesis in their respective hosts, FIV infection of cats

proved to be a useful animal model to test antiviral therapies and experimental vaccines against lentiviral infections<sup>1</sup> (for review see ref. 2). Furthermore, as FIV infection occurs in domestic cats worldwide<sup>3</sup>, the development of a vaccine is also of veterinary importance.

Since the discovery of FIV, many attempts have been made to develop a safe and effective vaccine. However, to date only four groups have reported the development of an experimental vaccine capable of protecting cats from challenge infection, albeit only against closely related challenge viruses. These successful vaccines consisted either of fixed FIV infected cells or of whole inactivated virus<sup>4-8</sup>. Two different cell lines were used to propagate the virus, used for vaccination. The first is the FL4 cell line, persistently infected with an American virus strain (FIV/Petaluma)<sup>4-7</sup>, the second is the MBM cell line

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infected with an Italian FIV isolate (FIV/M2)<sup>8</sup>. The mechanism by which these vaccines induced protective immunity remains unclear. This is further heralded by the observation that both approaches yielded contradictory data concerning possible correlates of protective immunity. Yamamoto *et al.* and Husic *et al.* reported that virus neutralising (VN) antibodies seem to play a major role in the observed protection, as indicated by the observation that most of the protected cats had developed high VN antibody titres against the homologous FIV/Petaluma strain<sup>9,10</sup>. Furthermore, naive cats, passively immunised with pooled sera from vaccinated cats, proved to be protected from subsequent homologous challenge<sup>9</sup>. In contrast, Matteucci *et al.* found that cats were protected against homologous challenge in the absence of detectable VN serum antibodies<sup>11</sup>. Data collected by Bishop *et al.* also could not show a role of VN antibodies in the protective responses raised by the FL4/FIV-Petaluma fixed cell vaccine<sup>12</sup>. Interestingly, the latter group suggested that protection was not achieved against latent infection, since the vaccinated cats became FIV-positive when monitored for a longer period of time, i.e. 50 weeks post challenge<sup>12</sup>.

Unfortunately, all other attempts to develop FIV vaccines, including experimental subunit vaccines containing either single or combinations of FIV proteins, were of limited success<sup>11-13</sup>. The majority of these studies used FIV envelope glycoproteins produced using different expression systems and administered with different adjuvants. Although no protection was induced, in some cases a lower cell associated virus load was observed upon challenge infection<sup>11,13</sup>. Also, vaccination with a synthetic peptide, containing a neutralising antibody inducing epitope, failed to protect cats from challenge infection<sup>14</sup>.

Recently, we reported that vaccination of cats with envelope glycoprotein subunit vaccines that induced high titres of VN plasma antibodies accelerated the development of viraemia upon FIV challenge<sup>12</sup>. These VN antibodies could be detected with a Crandell feline kidney (CrFK) cell line adapted virus but not with primary FIV isolates. In addition, the observed enhancement could be transferred to naive cats via plasma pools from the vaccinated animals and was therefore probably mediated by specific antibodies. In the present study we extend these observations by testing a series of experimental iscom based FIV subunit vaccines for their ability to induce VN antibody responses detectable against a FIV strain adapted to replicate in CrFK cells and against primary FIV isolates. Subsequently the cats were challenged with a highly homologous virus strain and monitored for the development of viraemia.

## MATERIALS AND METHODS

## Tissue culture

Feline peripheral blood mononuclear cells (PBMC) and thymocytes were isolated from an 8-week-old specific pathogen free (SPF) cat, as described previously<sup>15</sup>. These cells were stimulated with concanavalin A (ConA) at a concentration of 5  $\mu$ g ml<sup>-1</sup> in culture medium (CM) which consists of RPMI 1640 (GIBCO, Gaithersburg, MD) supplemented with

penicillin (100 IU ml<sup>-1</sup>), streptomycin (100  $\mu$ g ml<sup>-1</sup>), L-glutamine (2 mM), 2-mercaptoethanol (2  $\times$  10<sup>-5</sup> M) and 10% fetal bovine serum (GIBCO). After 3 days of stimulation the cells were washed and cultured further in CM supplemented with recombinant interleukin-2 (rIL-2) (200 IU ml<sup>-1</sup>) (Eurocetus). The cells were cryopreserved and frozen at -135°C until further use. A clone of Crandell feline kidney cells (CrFK 1D10 cells) susceptible to infection with FIV, was kindly provided by N. Pedersen and grown under standard conditions<sup>16</sup>.

## Viruses

FIV AM19 was isolated from PBMC of a naturally infected cat<sup>15</sup>. The virus was subsequently propagated in ConA and rIL-2 stimulated PBMC. After FIV antigen was detected in the culture supernatant by ELISA<sup>17</sup>, virus was filtered through a 220 nm pore-size filter, aliquoted and stored at -135°C. This FIV stock was titrated *in vivo* and subsequently used as challenge stock as described previously<sup>12</sup>.

FIV AM6c was initially isolated from PBMC of a naturally infected cat and subsequently adapted to replicate in CrFK 1D10 cells and titrated *in vitro* as previously described<sup>18</sup>.

FIV 19k1 is a molecular clone, directly derived from bone marrow cells of a naturally infected cat<sup>15</sup>. FIV AM6c and FIV 19k1 were used in the two VN assays (see below), amino acid sequence homology of the Env protein of these viruses is 90.1% (K. Sichelin, unpublished results).

## Preparation of candidate FIV vaccines

Preparation of the FIV-iscoms was carried out with virus obtained from culture supernatant of FIV AM6c infected CrFK cells, which was concentrated with a Provario 100 kDa crossflow filter (J & M Separations). Subsequently, the concentrate was centrifuged for 2 h at 100000g at 10°C on two layers of sucrose of 10% and 50%, respectively. The interphase was harvested and dialysed against PBS for 16 h. With purified FIV virions thus obtained, iscoms were prepared as described previously<sup>19</sup>. In brief, the proteins of the virions were solubilised in 2% MEGA-10 (Boehringer Mannheim) and mixed with the lipids cholesterol and phosphatidylethanolamine (Sigma) and Quil A (Spikoside; ISCO-TEC) at a ratio of 1:1:5 (w/w) in the presence of 0.2% MEGA-10. After ultrasonication for 30 sec the mixture was incubated for 1 h at 37°C, followed by dialysis against PBS at room temperature for 16 h and an additional 24 h at 4°C.

vGR657x15-iscoms were prepared with recombinant vaccinia virus (rVV) expressed envelope glycoprotein of FIV AM19 from which the cleavage site between the surface and transmembrane protein was deleted (vGR657x15) as previously described<sup>19</sup>.

To prepare FIV-Gag-iscoms, FIV Gag was expressed with maltose binding protein (MBP) as a fusion protein (MBP-Gag). The gag gene of the 5' subclone of 19k1<sup>15</sup> was amplified by PCR, using the following primers (based on the FIV/Petaluma sequence, published by Talbott *et al.*<sup>20</sup>): primer 1, 5'GGACGAATTCATGGGGATGGACCGGGC3'; primer 2, 5'CCGAGAATTCTTACAAATCCAATAG-TTTCT3'. These primers contain an EcoRI restriction

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site (**bold, underlined**) in order to clone the amplified *gag* gene into the *Eco*RI site of the pMal-CRI vector (New England Biolabs), thus constructing pMal-Gag by which *gag* is expressed linked to MBP by a sequence sensitive for cleavage by complement factor Xa. pMal-Gag was cotransfected with pUBSS20 in *E. coli* BL21-lys to enhance expression of the fusion protein<sup>21</sup>. MBP-Gag was isolated by sonication of the bacteria and subsequently purified by column chromatography using an amylose column. Upon incubation of the fusion protein on the amylose column and subsequent washing of the column it was digested with factor Xa (New England Biolabs) to isolate the Gag protein. Gag was subsequently coupled to palmitic acid to facilitate incorporation of the Gag protein into iscoms, as previously described<sup>22</sup>.

Control iscoms were prepared with culture supernatant of uninfected CrFK cells that was concentrated with a Provario 100 kDa crossflow filter. Subsequently, 5 mg of total protein was solubilised in 2% MEGA-10 and used for the preparation of iscoms as described above.

SIV envelope glycoprotein iscoms (SIV-Env-iscoms), used as controls in this study, were provided by E. Hulskotte and had been prepared following a procedure similar to that used for the preparation of vGR657x15 iscoms<sup>23</sup>.

#### Vaccination procedure and challenge

Five groups of six and one group of five (group 3) specified pathogen free (SPF) cats were vaccinated subcutaneously three times with candidate vaccines and control preparations according to the following schedule: group 1, FIV-iscoms; group 2, FIV-iscoms+vGR657x15-iscoms; group 3, vGR657x15-iscoms+FIV-Gag-iscoms; group 4, CrFK-iscoms; group 5, SIV-Env-iscoms; group 6, PBS.

Cats vaccinated with either FIV or SIV-Env-iscoms received 10 µg of the respective proteins per dose. Cats vaccinated with FIV-iscoms or FIV-Gag-iscoms received 10 µg of Gag protein per dose. Protein doses were determined by ELISA as previously described<sup>12</sup>. CrFK-iscom vaccinated cats received the same amount of total protein as was present in the FIV-iscoms. Cats were vaccinated at 0, 4 and 10 weeks. Two weeks after the last vaccination the cats were challenged intramuscularly with 20 CID<sub>50</sub> of cell free FIV AM19. Plasma samples and PBMC were collected every 2 weeks, starting 2 weeks prior to challenge infection, as previously described<sup>15</sup>.

#### Serology

Plasma samples of the cats were tested for their reactivity with bacterial fusion proteins containing different regions of the FIV envelope protein by ELISA as previously described<sup>24</sup>. In brief, 96-well microtitre plates were coated overnight at 4°C with the bacterial fusion proteins at 100 ng per well. After washing with PBS/0.05% Tween 20, the plates were blocked with 5% goat serum in PBS/0.05% Tween 20 for 1 h at 37°C, followed by another wash procedure. Hereafter, the cat sera were diluted 1:500 in PBS/0.05% Tween 20/5% goat serum and incubated for 1 h at 37°C. Upon washing, the plates were incubated with horseradish peroxidase labelled goat anti-cat

serum (Cappel) in PBS/0.05% Tween 20/5% goat serum for 1 h at 37°C after which horseradish peroxidase activity was detected. Subsequently, the optical density of the samples was measured at 450 nm in an ELISA reader (Titertek, Multiscan Plus).

Antibodies directed against p24 and p17 Gag proteins were detected with a commercially available ELISA (catalogue number F1002-AB01; European Veterinary Laboratory, Woerden, The Netherlands). The titres found are expressed as:

$$OD_{450} (\text{plasma}) \times \text{dilution} / 3 \times OD_{450} (\text{negative plasma})$$

#### Virus neutralisation assays

VN antibodies present in plasma of the cats were measured in two different VN assays. The first assay is based on the inhibition of infection of CrFK 1D10 cells with an FIV strain adapted to replicate in these cells, FIV AM6c<sup>12</sup>. VN plasma antibody titres were defined as the reciprocal of the highest dilution of a plasma sample still inhibiting FIV replication as measured by the detection of viral antigen in the culture supernatants of the CrFK cells by ELISA<sup>17</sup>. The second assay is based on the inhibition of infection of feline thymocytes with molecular clone FIV 19k<sup>25</sup>. VN was considered positive when the RT activity was below two times the background level, measured with uninfected cells.

#### Cell associated virus load

Cell associated virus load was measured by an infectious centre test, essentially as previously described<sup>12</sup>. In short, serial dilutions of PBMC isolated from the cats ( $1 \times 10^3$ ,  $3 \times 10^3$  and  $1 \times 10^4$  cells) were prepared and subsequently co-cultured with stimulated PBMC from an SPF cat in eight wells. After 3 weeks culture supernatants were screened for presence of FIV antigen by ELISA and the *in vivo* cell associated virus load was calculated using the assumption that one infected cell gave rise to antigen production in an FIV antigen positive well after co-cultivation with PBMC from an SPF cat when one or more of the eight wells were negative for FIV antigen production.

#### Statistics

Considering the low number of cats in each group nonparametric tests were used for statistical analysis of the data. For tests between more than two groups the Kruskal-Wallis (K-W) test was applied. For tests between two groups the Mann-Whitney (M-W) test was used. Paired data were analysed with the Wilcoxon test. Since multiple tests were performed the  $\alpha$  level of significance was conservatively adjusted to  $\alpha = 0.05/3 = 0.017$  (i.e.  $P$  values below 0.017 are assumed significant).

## RESULTS

#### Development of FIV-specific antibody responses upon vaccination

In cats immunised with FIV-iscoms (group 1), no or relatively low antibody responses to most of the different regions of the envelope protein could be demonstrated (Figure 1). In contrast, cats that had

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Group number	cat no	SU1	SU2	SU3	SU4	SU5	SU6	TM1	TM2	TM3
1	49							■■■	■■■	
	71									
	76			■						
	90							■■■	■■■	
	50			■				■■■	■■■	■■■
	31									■■■
2	48	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■
	75	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■
	97	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■
	108	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■
	93	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■
	81	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■
3	57	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■
	95	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■
	113	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■
	109	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■
	61	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■	■■■
4	54									
	80									
	87									
	102									
	91									
	21									
5	43									
	83									
	86									
	110									
	H333									
	17									
6	42									
	68									
	104									
	103									
	25									
	27									

Figure 1 Levels of plasma antibodies present in cats of the different vaccination groups at the day of challenge, against bacterial fusion proteins containing different regions of the FIV envelope glycoprotein<sup>24</sup>. Plasma samples were tested at a dilution of 1:500. No symbol,  $OD_{450} < 0.6$ ; ■,  $0.8 < OD_{450} < 1.2$ ; ■■,  $OD_{450} > 1.2$ .

received vGR657x15-iscoms, both in combination with FIV-iscoms and FIV-Gag-iscoms (groups 2 and 3), developed a relatively high antibody response to most of these regions, compared with the cats of group 1. In plasma of cats of control groups 4, 5, and 6, virtually no antibodies against these Env regions were detected.

In the plasma samples of all cats in groups 2 and 3, collected at the day of challenge, significant VN antibody levels could be demonstrated when tested in the CrFK cell based VN assay ( $P \leq 0.004$ ), with titres ranging from 20 to 640 and a median VN titre of 40 and 160, respectively (Figure 2). In contrast, the cats of group 1, vaccinated with FIV-iscoms, did not develop significant VN antibody levels ( $P \geq 0.32$ ), although one cat did develop a VN antibody titre of 160. Moreover, the VN antibody titres of groups 2 and 3 are significantly higher than those of group 1 ( $P = 0.019$  and  $P = 0.012$ , respectively). None of the plasma samples collected at the day of challenge, exhibited VN activity when tested in the thymocyte based VN assay (not shown). None of the cats in the control groups showed any VN activity in either of the assays at the day of challenge (Figure 2).

All cats in groups vaccinated with FIV derived proteins developed significant levels of Gag specific plasma antibodies after two immunisations ( $P \leq 0.006$ ) (Figure 3). At the day of challenge, after three immunisations, cats of group 1, vaccinated with FIV-iscoms, exhibited the highest levels of Gag-specific plasma antibodies with a median titre of about 79000. At this time, the median titres of Gag-specific plasma antibodies in cats of groups 2 and 3, vaccinated with vGR657x15-iscoms+FIV-iscoms and vGR657x15-iscoms+Gag-iscoms respectively, were about 40000 and 57000. Although the Gag-specific plasma antibody titres of the cats of group 1 tended to be higher than those of groups 2 and 3, titres did not significantly differ compared with each other ( $P \geq 0.04$ ). No

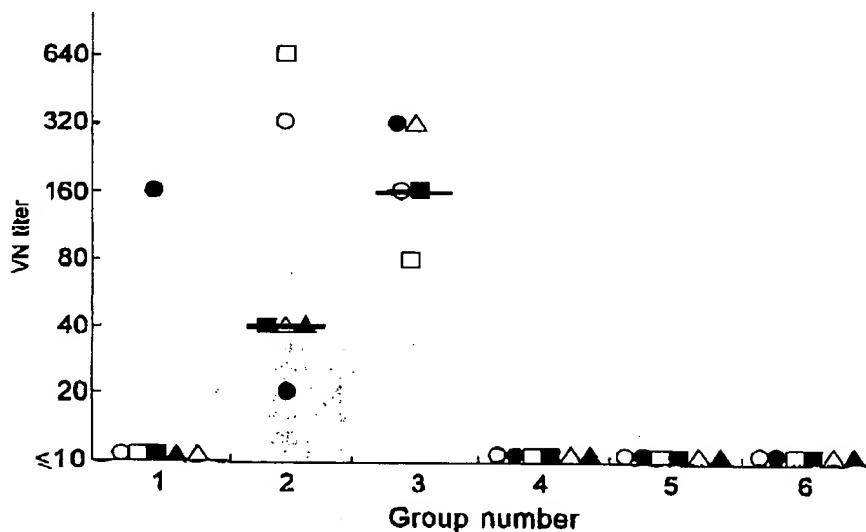
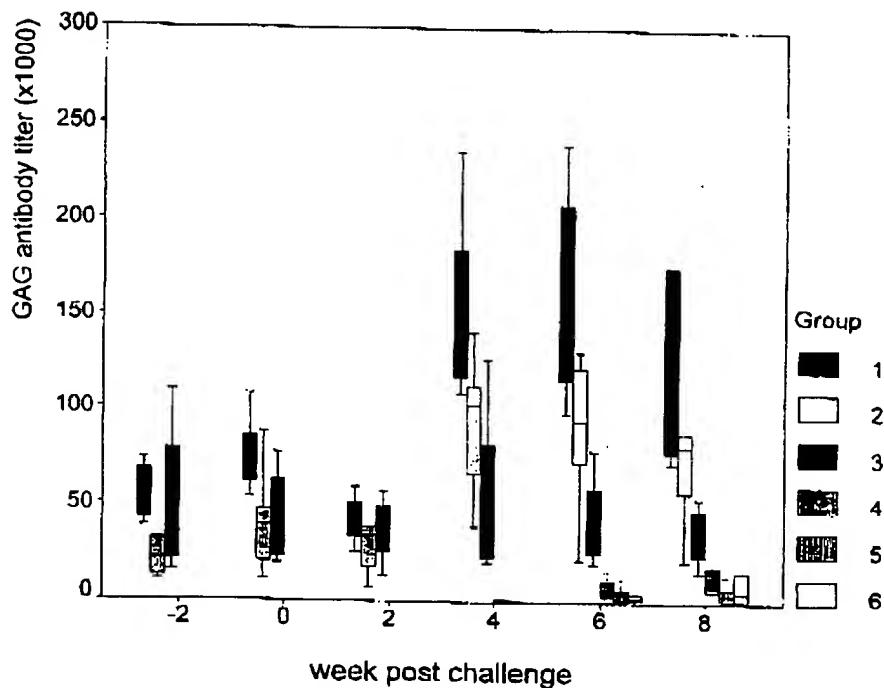


Figure 2 Virus neutralising antibody titres measured at the day of challenge using the CrFK cell based VN assay. Each symbol represents the result of one individual cat. The horizontal bars represent the median VN titre in one group of cats. When the median titre of a group of cats was 0, no horizontal bar is shown.

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**Figure 3** Development of Gag-specific plasma antibodies in the different vaccination groups upon vaccination and subsequent challenge infection. Median Gag-specific plasma antibody titres of each group are presented as horizontal bars within the boxes

Gag-specific plasma antibodies were detected in cats of the three control groups.

#### Development of Gag-specific plasma antibody titres after challenge infection

Four weeks after challenge infection with FIV AM19, a clear increase was observed in the Gag specific plasma antibody titres in cats of groups 1 and 2, which were vaccinated with FIV-iscoms alone, or in combination with recombinant Env-iscoms (median titres of 146000 and 102000, respectively) (Figure 3). The increase of Gag specific plasma antibody titres in group 3 was less pronounced (median titre of 60000), although the lower increase of the Gag specific plasma antibody titres in this group was just at or above the level of significance compared with groups 1 and 2 ( $P = 0.018$  and  $P = 0.04$ , respectively). In cats of the three control groups, Gag-specific plasma antibodies could not be detected until 6 weeks after challenge, with the exception of one cat in group 4 that showed a low titre from week 4 onward.

#### Cell associated virus loads after challenge infection

Two weeks after challenge infection, FIV infected PBMC could be detected in two cats of group 1, with virus loads of 13 and 42 infected cells per  $10^6$  PBMC, five cats of group 2, with virus loads ranging from 13 to 106 infected cells per  $10^6$  PBMC, two cats of group 3, with virus loads of 13 and 27 infected cells per  $10^6$  PBMC, and three cats of control group 4, with virus loads ranging from 76 to 168 infected cells per  $10^6$  PBMC (Figure 4(A)). At this time no infected cells could be detected in cats of groups 5 and 6. Statistical analysis revealed that only the cats of group 2 had

developed significant numbers of FIV-infected PBMC at this time point compared with control groups 5 and 6 ( $P = 0.007$ ). However, the virus load of cats of group 2 did not differ significantly from that found for the cats of control group 4 ( $P = 0.74$ ).

At 4 weeks after challenge, all cats had become viraemic, with virus loads ranging from 42 to 1000 infected cells per  $10^6$  PBMC, and there were no significant differences in the average virus loads between the groups ( $P \geq 0.08$ ) (Figure 4(B)).

#### DISCUSSION

In the present study we have shown that, with different iscom-based FIV subunit vaccines, high titres of both Gag- and Env-specific plasma antibodies can be induced. The highest levels of envelope specific plasma antibodies were observed in cats of groups 2 and 3, which could be expected since these cats had received vaccine preparations that contained purified envelope glycoprotein incorporated into iscoms. In contrast, cats of group 1 had received iscoms containing purified FIV derived from infected CrFK cells, in which less envelope glycoprotein may be present.

Although VN activity was induced, albeit only significantly in groups 2 and 3 which were vaccinated with vaccine preparations containing purified vaccinia expressed Env incorporated into iscoms, none of the animals proved to be protected from challenge infection with a largely homologous FIV strain. In this context, it is important to note that VN antibodies could only be demonstrated in the CrFK cell based assay, using a FIV strain adapted to replicate in this cell line. In contrast, no VN activity was demonstrated in the primary feline thymocyte based assay, using a

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largely homologous virus strain (*env* gene sequence homology  $\geq 95\%$ ) that was not adapted to replicate in CrFK cells. Adaptation to replication in the CrFK cell line apparently alters the sensitivity of FIV to VN antibodies *in vitro* as we and others noted previously<sup>12,26,27</sup>. In HIV infection of humans, we and others described a similar phenomenon: Antibodies that efficiently neutralise T cell line adapted HIV-1 strains, fail to neutralise or even enhance infectivity of primary HIV-1 isolates in primary lymphoid cell cultures<sup>28,29</sup>.

We previously reported the results of studies with FIV subunit vaccines, in which the induction of VN antibodies, only detectable in the CrFK cell based VN assay, predisposed for accelerated viraemia rather than for protection upon FIV challenge<sup>12</sup>. In the experiments reported here, cell associated virus loads, measured two weeks after challenge infection also indicated a tendency toward accelerated viraemia in the cats of group 2, vaccinated with FIV-iscoms and vGR657x15-iscoms, compared with control groups 5 and 6 ( $P = 0.007$ ). However, three out of six cats of group 4, vaccinated with iscoms prepared with the supernatant of uninfected CrFK cells, also proved to be viraemic 2 weeks after challenge, when the SIV-Env-iscoms and PBS inoculated groups (5 and 6) were still not viraemic (Figure 4a). Moreover, the

respective virus loads of groups 2 and 4 were not significantly different at this time point ( $P = 0.74$ ). Since the cats of group 4 had been immunised with a vaccine preparation devoid of FIV proteins, the accelerated onset of viraemia observed in this group could not be attributed to a FIV specific antibody response. These results imply that, if in this group antibody mediated enhancement had indeed taken place, antibodies to cell derived components in the virus membrane would have caused this phenomenon.

Addition of bacterially expressed Gag protein incorporated into iscoms in the vaccine preparation seemed to counteract the enhancing effect induced by vaccination with purified FIV envelope glycoprotein containing vaccines. This is best illustrated by the fact that only two of five cats of group 3, vaccinated with vGR657x15-iscoms and Gag-iscoms, had become viraemic 2 weeks after challenge. In a parallel study in which the same challenge infection was used, all six cats vaccinated with vGR657x15-iscoms alone had become viraemic at this time and the cell associated virus loads of all the individual cats of that experiment were much higher than those of the two cats of group 3<sup>12</sup>. It is interesting to note that the two cats in which FIV infected PBMC could be demonstrated, had developed the lowest anti-Gag response of this group at the day of challenge (not shown). Since it cannot be expected that anti-Gag antibodies would have contributed to the observed protective effect, it may be speculated that it was mediated by a Gag-specific T cell response.

Taken together, the differences in outcome between the few series of successful vaccinations versus the large number of unsuccessful FIV vaccination experiments, including those presented in this study, indicate that mechanisms leading to enhanced infectivity may interfere with the induction of protective immunity. Therefore, the rational development of vaccines against lentiviruses including FIV and HIV, should not only focus on the identification of correlates of protective immunity, but also on correlates of vaccine induced enhanced viral infectivity.

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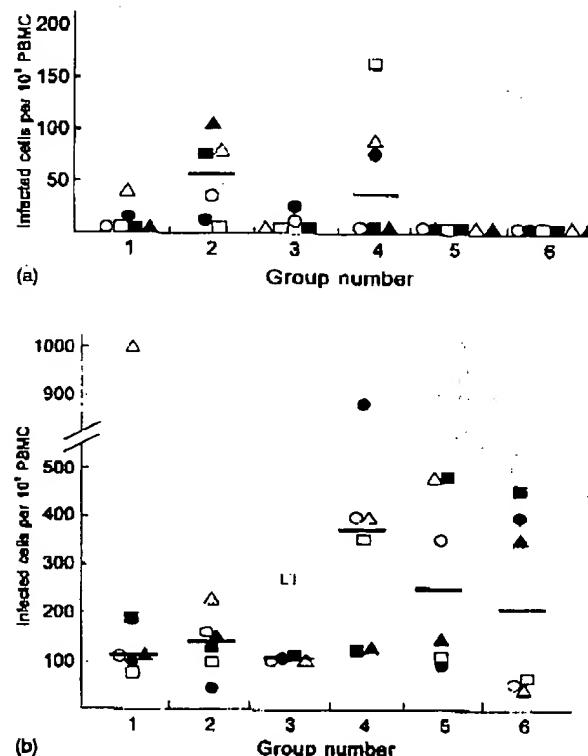


Figure 4 Cell associated virus loads after challenge infection. The number of FIV infected cells per  $10^6$  PBMC collected from cats at 2 (a) and 4 (b) weeks after challenge were determined in the infectious centre test. Each symbol represents the result of one individual cat, median numbers of infected cells in a group of cats are depicted as a bar. When the median virus load in a group of cats was 0, no horizontal bar is shown.

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